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ORIGINAL ARTICLE

Eukaryotic translation initiation factor 4-γ, 1 gene mutations are rare in Parkinson's disease among Taiwanese

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Background/Purpose: Parkinson's disease (PD) is the second most common neurodegenerative disorder worldwide. Although idiopathic PD accounts for most of the cases, several genetic mutations have been found to cause PD. Mutations in the eukaryotic translation initiation factor 4-γ, 1 (*EIF4G1*) gene have been identified since 2011, which were reported to be associated with PD among Caucasians in subsequent research. However, this observation was not consistent. The contribution to other ethnic groups remains limited, with < 1% of sporadic cases. We conducted a case–control study to analyze if *EIF4G1* is a risk factor for PD patients in Taiwan.
Methods: There were 595 PD patients and 600 controls without neurological diseases enrolled in this study. Four reported mutations—A502V (c.1505C>T), G686C (c.2056 G>T), R1197W (c.3589C>T), and R1205H (c.3614G>A)—were analyzed.
Results: There were no mutations found in either PD patients or controls.
Conclusion: This study indicates that the *EIF4G1* mutation is rare in Taiwan, which is consistent with other reports from Asia. Ethnicity could have a great influence on *EIF4G1* in PD. Further large scale studies are warranted to evaluate the association of PD and *EIF4G1* gene.

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder with cardinal features of resting tremor, rigidity, bradykinesia, and postural instability.[1] Idiopathic PD accounts for most cases, but ~5–10% of PD could be caused by gene mutation.[2] Several of these Mendelian-transmitted mutations have been identified such

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as *SNCA*, which accounts for *PARK1* and *PARK4*,[3] *PRKN* for *PARK2*,[4] *PINK1* for *PARK6*,[5] *DJ-1* for *PARK7*,[6] *LRRK2* for *PARK8*,[7] and *ATP13A2* for *PARK9*.[8] In 2011, Chartier-Harlin et al[9] identified a new missense mutation R1205H (c.3614G>A) in the eukaryotic translation initiation factor 4-γ, 1 (*EIF4G1*) gene in one French family. Subsequent sequence and genotype analysis identified *EIF4G1* A502V (c.1505C>T), G686C (c.2056 G>T), S1164R (c.3490A>C), and R1197W (c.3589C>T) substitutions in affected patients with familial parkinsonism and idiopathic Lewy body disease but not in control individuals.[9] Later, several *EIF4G1* mutation/variants were investigated for association with PD.[10–23] However, the results did not confirm these genetic associations.

EIF4G1, a protein scaffold subunit of the translation initiation complex, binds the ribosomal 40S. A decrease of EIF4G1 level in cells results in a reduction of overall protein synthesis linked to nutrient perception.[24] Pathogenic EIF4G1 substitutions, A502V and R1205H, have been demonstrated to disrupt binding to EIF4E and EIF3E, respectively, and thus lead to mitochondrial dysfunction.[9] By contrast, overexpression of EIF4G1 protein has been implicated in cell proliferation as observed in some malignant disorders.[25] Furthermore, in a yeast model, a EIF4G1 ortholog (TIF4632) may reduce á-synuclein toxicity.[26] Taken together, this evidence suggests that EIF4G1 may play a role in PD regarding maintaining mitochondrial function and cell survival as well as protecting against neuron damage from accumulated á-synuclein.

In Taiwan, the link between *EIF4G1* variants and PD has not been investigated. Here we conducted the first *EIF4G1* genetic study in the Taiwanese population and tried to determine the frequency of *EIF4G1* variants among PD patients to clarify the association between *EIF4G1* variants and PD.

Methods

Patients

For this study, 595 patients diagnosed with PD were recruited from the neurology clinics of Chang-Gung Memorial Hospital, Taipei, Taiwan. Only the probands of each familial PD family were included in the study. The diagnosis of PD was based on the modified UK Parkinson's disease society brain bank clinical diagnostic criteria.[27] In addition, 600 unrelated adult volunteers without neurological diseases matched for age, sex, ethnic origin, and area of residence were recruited as controls. All participants gave informed consent for the study under a protocol (No. 101-2599A3) approved by hospital internal ethics and scientific boards.

Genotyping and data imputation

DNA was extracted from peripheral blood by standard protocols. Four mutations of the *EIF4G1* gene—A502V, G686C, R1197W, and R1205H—were investigated. These mutations, firstly found in 2011 by Chartier-Harlin et al,[9] were the most common screened mutations to be published in subsequent literature.[10–23] Although S1164R was also identified in 2011,[9] it was not included because there was no positive finding in these studies.[10–23]

The genotyping was performed by Sequenom MassARRAY platform with iPLEX gold chemistry (Sequenom, San Diego, CA, USA). By following the manufacture guide, the specific polymerase chain reaction (PCR) primer and extension primer sequences are designed with Assay Designer software package (version 4.0, Sequenom MassARRAY system). The primers used for PCR amplification are listed in Table 1. One μL of Genomic DNA sample (10 ng/μL) was applied to multiplex PCR reaction in 5 μL volumes containing 1 unit of Taq polymerase, 500 nmol of each PCR primer mix and 2.5 mM of each dNTP (PCR accessory and Enzyme kit, Sequenom). Thermocycling was at 94°C for 4 minutes followed by 45 cycles of 94°C for 20 seconds, 56°C for 30 seconds, and 72°C for 1 minutes, then 72°C for 3 minutes. Unincorporated dNTPs were deactivated using 0.3 U of shrimp alkaline phosphatase. The single base extension reaction was using iPLEX enzyme, terminator mix, and extension primer mix followed by 94°C for 30 seconds followed by 40 cycles of 94°C for 5 seconds, and five inner cycles of 56°C for 5 seconds and 80°C for 5 seconds, than 72°C for 3 minutes (iPLEX gold kit, Sequenom). After the addition of a cation exchange resin to remove residual salt from the reactions, 7 nL of the purified primer extension reaction was loaded onto a matrix pad of a SpectroCHIP (Sequenom). SpectroCHIPs were analyzed using a MassARRAY Analyzer 4 (Sequenom), and the calling by clustering analysis with TYPER 4.0 software (Sequenom).

Results

The mean age of 595 PD patients was 64.8 ± 10.2 years, and the mean age at onset of PD symptoms was 62.7 ± 11.3 years; 45% were women. The age at recruitment of 600 controls was 59.1 ± 12.7 years; 53% were women. Statistically, both groups demonstrated equal age of recruitment, age at onset, and sex distribution. There were 21 patients with PD family history and 120 patients with age at onset younger than 50 years. All familial PD patients have screened for *LRRK2* variants, which found *LRRK2* R1441H mutation in one patient. Additionally, two sporadic patients with age at onset at age < 50 years carrying *PARK2* deletions (Ex2-3del and Ex5del, respectively). Four selected mutations of *EIF4G1* described in the method were examined. Our study revealed that *EIF4GI* A502V, G686C, R1197W, and R1205H were all absent in both patients and normal controls. This finding indicated that these mutations are rare among PD patients in Taiwan.

Discussion

In 2011, *EIF4G1* gene was first described to be a possible gene accounting for late onset, autosomal dominant PD in Caucasian populations by Chartier-Harlin et al.[9] R1205H was identified initially in a French family with PD. The parkinsonian feature in this family was a relatively long course, mild symptom, and preserved cognition. R1205H was further investigated and found in seven PD families from USA, Canada, Ireland, Italy, and Tunisia.[9] Other *EIF4G1* variants including A502V, G686C, S1164R, and

Table 1 Primers for detection of the EIF4G1 A502V, G686C, R1197W, and R1205H variants.

Variants	Accession No.	Forward primer	Reverse primer	Unextension primer
A502V	rs111290936	ACGTTGGATGAGAGAGTACCCCTATTCCAG	ACGTTGGATGTCCAACCACACCTTACCTTG	CCTTGAGTGGCTGCT
G686C	rs112019125	ACGTTGGATGTCCATCCTTTGCCAACCTTG	ACGTTGGATGTTTACCCAGCCCCACTCAC	GGGGCGTGGGCCCCCAAGG
R1197W	rs113388242	ACGTTGGATGTCAGCAAGGAAGTGGAGGAG	ACGTTGGATGCATTCCTCTCCCAGACTCTC	TGGGAGCGGAGTAGAGAA
R1205H	rs112176450	ACGTTGGATGTGGAGGAGCGGAGTAGAGAA	ACGTTGGATGCATTCCTCTCCCAGACTCTC	GGAGCCTGAGGGGCTGC

R1197W were also found from the USA, France, and Poland PD families.[9] Among them, A502V and G686C were also found from idiopathic PD patients.[9] Functional analyses have shown that *EIF4G1* A502V and R1205H disrupt EIF4E or EIF3E binding, increasing their susceptibility to reactive oxidative species.[9]

Recently, a study from the French PD genetic study group found R1197W in a control individual, but not in PD patients, suggesting that R1197W may not be a causative mutation for PD.[10] In another study from central Europe, five PD cases carrying G686C mutation were identified, but this variant was also found in three normal controls.[11] Additionally, R1205H was present in healthy controls but not in cases in the same study.[11] After its first report in 2011,[9] R1205H has been identified in some patients from America via whole exome sequencing[13] but only one patient from a most recent large European cohort study.[12] By contrast, R1205C rather than R1205H was identified in one patient from southwest China.[21] Due to the conflicting results listed above, the importance of *EIF4G1* mutations in PD remains doubtful. The possible explanations for identification of mutants in controls include the possibility of development of PD in the future, incomplete penetrance, or the mutation conferring a risk for PD. The summary of previous reports about EIF4G1 is listed in Table 2.

From our study, *EIF4G1* mutations were not observed among PD patients and controls. The result was reminiscent of other studies in different countries such as China, Singapore, Japan, South Africa, India, and Italy, all of which showed negative findings.[14–20,22] Possible reasons could be: first, *EIF4G1* gene mutations could be mutation in PD, so study of more PD patients is warranted. Secondly, the first reported cases were from Caucasians and subsequent positive results were mostly European or American. Only one Chinese PD patient with *EIF4G1* R1205C mutant was found in a recent study,[21] and other mutations were not observed among other Asian populations.[14–17,22] Ethnicity may have a significant influence on the negative results. Furthermore, it may also be possible that western and eastern patients had different prevalent mutation variants, and thus it is difficult to find *EIF4G1* mutation in the Eastern population if only screening previously identified mutations. For example, T355I (c.1064C>T) was recently been found in one Asian descent[23] and also in African–American people found in the European American population.[13] The important role of ethnicity in PD had been discussed in previous study.[28] A good example is leucine-rich repeat kinase 2 gene (*LRRK2*) G2019S variant is the most common mutation for Caucasian PD patients, but it is rare for Asians.[28–32] By contrast, G2385R variant is a genetic risk factor for Asian PD patients but very rare for Caucasian patients.[28,33–37] Besides, the microtubule-associated protein tau gene r393152 variant on the locus has been shown to be a risk factor for PD in Caucasians as compared to Asians.[28] Genetic variants of bone marrow stromal cell antigen gene and *PARK16* also have an ethnic difference.[28]

The limitation of this study is that we only screened A502V, G686C, R1197W, and R1205H rather than sequencing all exons and exon–introns of *EIF4G1* gene. It would be possible to miss novel mutations in our study.

Table 2 Summary of *EIF4GI* studies. The table listed the year of published literature and screened method. The result of variants are also listed.

Published year	Authors	Screened method	Positive result from patients	Number of PD patients (F:S)[b]	Ethnic population	Refs
2012	Schulte et al	Sequencing exons & exon–intron boundaries A502V, G686C, S1164R, R1205H	Multiple, include G686C	376 975 (557:418)[a]	European	11
2012	Lesage et al	Sequencing exons & exon–intron boundaries	Multiple, include G686C	251 (251:0)	European	10
2012	Tucci et al	Sequencing exon 8 & exon 22	P486S	150 (150:0)	European	20
2013	Nuytemans et al	Whole exome sequencing	Multiple, include R1205H	213 (25:188)	Caucasian, non-Hispanic/ Latino descent	13
2013	Li et al	Sequencing exons & exon–intron boundaries T161A, M432V, R1205H	T161A, M432V	29 (29:0) 503 (0:503)	Chinese	14
2013	Zhao et al	Sequencing exons & exon–intron boundaries M432V, P693S	M432V, P693S	96 (75:21) 791	Chinese Mixed Asian races	15
2013	Yuan et al	502V, R1205H	no	425 (119:306)	Chinese	16
2013	Sudhaman et al	Sequencing coding exons R1205H	no	15 (15:0) 305 (54:251)	Indian	22
2013	Chen et al	R1205H	R1205C	609 (37:572)	Southwest Chinese	21
2013	Siitonen et al	Sequencing coding region	T355I, R1172_D1174del	168	Caucasian Asian African American American Indian Pacific Islander Other	23
2014	Nishioka et al	Sequencing coding exons A502V, R1205H	No	95 (95:0) 224 (43:181)	Japanese	17
2014	Blanckenberg et al	R1205H	No	418 (0:418)	Caucasian non-Afrikaner Caucasian Afrikaner Mixed ancestry African Indian	18
2014	Gagliardi et al	R1205H	No	250 (250:0)	European	19
2015	Huttenlocher et al	Sequencing coding region A502V, R1205H	Multiple, include R1205H	95 (95:0) 2051 (0:2051)	European	12

[a] There were 376 patients included in group of sequencing exons and exon–intron boundaries.
[b] F:S = familial:sporadic.

Although there was no *EIF4G1* A502V, G686C, R1197W, and R1205H mutation found in our study, this observation does not exclude other variants in the *EIF4G1* gene that may cause PD in Taiwan. Further investigations including whole gene sequencing and recruiting a large sample size of patients with PD from diverse populations, as well as studies of *EIF4G1* expression and in scaffold function, are required to evaluate the association between PD and *EIF4G1* gene.

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